Modulating Effect of Peppermint and Eucalyptus Essential Oils on vVND Infected Chickens

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ABSTRACT

Treatment of broiler chickens with the peppermint and eucalyptus essential oils blend improved protection and productive performance as compared with the infected positive control birds. It decreased total mortality, increased final body weight, and decreased final feed conversion ratio (FCR) (P≤0.05). The peppermint and eucalyptus essential oils blend had a positive effect on macroscopic and microscopic lesion scoring and enhanced HI and ELISA titers against Newcastle disease virus vaccine, as compared to their untreated control groups (P≤0.05). In conclusion: the peppermint and eucalyptus essential oils blend used in the present investigation significantly controlled vVND infection where it has immunomodulatory effect and positively evoked the immune response against this very hot challenging virus which is of great devastating economic impact on poultry industry.

INTRODUCTION

Herbal products and their oil extracts have been used for natural therapy as pharmaceuticals; however, only in recent years; aromatic plants and their extracts were introduced to the animal feeding (Awaad et al., 2014). Further complications arise because herbal products and their oil extracts feed additives may vary widely with respect to botanical origin, processing, and composition. Most studies investigated blends of various active compounds and reported their effects on production performance rather than the physiological impacts (Renata et al., 2012; Lee et al., 2013). Eventually; immunomodulated components strengthen the defense and immune mechanisms of the body and currently usable for stimulating the non-specific immune responsiveness in both the human and Veterinary Medical practice (Awaad et al., 1999). Peppermint and eucalyptus essential oils blend proved to have an immunostimulant effect on the humoral and cell mediated immune response against Newcastle disease in chickens (Awaad et al., 2009a). Their utility in modulating the immune response of immunocompromised birds after infection with infectious bursal disease virus (IBDV) and/or vaccination against IBDV as compared with untreated control groups was also evident (Awaad et al., 2009b).

The present trial was dedicated to determine the possible modulating effects of usage of peppermint and eucalyptus essential oils in emulsifiers on broiler chickens infected with velogenic Newcastle disease virus (vVND).

MATERIALS AND METHODS

Essential volatile oils blend: Peppermint and eucalyptus essential volatile oils in emulsifiers blend produced commercially under the trade name “Mentofin®” by EWABO Co., Germany was used. Mentofin consists of Cineol (42.2%), limonene (3.5%), L-menthol (48.7%), phellandrene (0.5%), a-pinene (1.0%), b-pinene (0.3%), and terpineol (0.3%).

Challenging Newcastle disease virus strain (vVNDV): The virus was isolated in 10 day-old chicken embryos and identified as NDV using PCR (Viljoen et al. 2005) (Fig.1). Sequence of PCR product proved to be similar to genotype VII, Chicken/China/SDWF07/2011-acc.no.: JQ015296 (Fig.2). The velogenicity of vVNDV was monitored by intramuscular inoculation of 10 birds (28 day-old broiler chickens free from antibodies to ND) with a dose of 10^6.8 EID50/ml/bird which resulted in 100% mortality.

Experimental design: One day-old Arbor Acres broiler chickens (n=300) were used in this study. They were allotted into 5 equal groups (1-5) consisting of 60 each. Chickens of all groups have been assigned into 3 equal
replicates (20 each) which kept in isolators with strict isolation measures. The birds had free access to water and fed on a commercial ration ad libitum. Experimental birds of groups 2, 3 and 4 were vaccinated against different diseases according to the vaccination programs usually adopted in Egyptian chicken broiler farms. Chickens of groups 1 and 5 were kept as a control groups without ND vaccination.

Chickens of groups 1, 3 and 5 were treated with peppermint and eucalyptus essential volatile oils in emulsifiers blend via both oral and spray routes. Oral treatment via drinking water was carried out at several intervals: 4-7, 16-18 and 25-39 days of age at a dose level of 0.25 ml/l for 12 hours/day. While Spray treatment carried out at 3, 7, 12, 13, 15,16, 19, 23, 27, 31, 35 and 39 days of age at a dose level of 0.1ml/20 ml water/10 birds using fine spray (< 3 μm diameter) as recommended by the producer. The birds of groups 2 and 4 were kept without treatment. At 28th day of age; 2 birds were randomly chosen for each replicate (4 birds/group) of groups 4-6 and infected intramuscularly with a dose of 10^6 EID₅₀/ml/bird of vVND virus and kept with other pen mates for contact infection. The birds of group 5 were kept without infection as negative controls. Infected birds groups kept under close observation for further 14 days post infection (PI) for survival rate and macroscopic lesion scoring of dead as well as sacrificed birds at the end of observation period. At 1st day of age blood samples were collected from randomly selected 12 birds for determining mean maternal antibody titer to NDV. In addition, 36 birds/group (12/replicate) were collected at 7, 14, 21 and 28 days of age and tested using ELISA (Idexx, ME, USA) and hemagglutination inhibition test (HI) (Swayne et al., 1998). At 35 days of age; 3 randomly chosen contact infected chickens/group of all infected experimented groups were sacrificed and samples of cecal tonsils, lungs and trachea were collected and subjected to histopathological examination for microscopic lesion scoring. The remaining chickens were subjected to chicken performance response variables.

### Statistical analyses

One-way analysis of variance was adopted using SAS software general liner models procedure (SAS Institute, 2004). The main factor was Mentofin supplementation as a mean effect. Mean values assessed for significance using Duncan’s multiple range tests. The following model was used for data analysis: \[ Y_{ij} = \mu + a_i + e_j \] Where; \( Y_{ij} \); The 3rd observation of the i-th dose of Mentofin, \( \mu \): Overall mean, \( a \): Effect of dose of Mentofin (1=1, 2), \( e \): Unexplained error. Statements of statistical significance based upon P≤0.05.

### RESULTS

High survival rate (100%) was observed in the negative control group (Treated/Vaccinated/Non-challenged), while 100% mortality was recorded in the positive control group (Untreated/Non-vaccinated/Challenged). Concomitant use of volatile oils with ND vaccination in group 3 (Treated/Vaccinated/Challenged) gave the superior results in protection against vVND challenge (80%) (Table 1). The survival rate against vVNDV challenge reached 18.33% and 61.67% in group 1 (Treated/Non-vaccinated/Challenged) and group 2 (Untreated/Vaccinated/Challenged) respectively. The postmortem lesion scoring varied between groups and was higher in group 4 (Untreated/Non-vaccinated/Challenged). Essential oils supplementation significantly improved performance parameters (body weight and FCR) of the treated groups (Table 2).

Mean ELISA titers were significantly higher at all studied intervals in ND vaccinated/volatile oils treated groups 3 and 5 as well as non-vaccinated/volatile oils treated group 1 vs. positive control group 4 (P<0.05) (Table 3). HI titers of ND vaccinated/essential oils treated groups (3 and 5) gave the higher significant increase over those of groups 1 and 4 at all studied intervals (P<0.05) (Table 4).

### Table 1: Results of vVNDV challenge of different broiler chicken groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of survived birds/ Total No. of birds*</th>
<th>Mortality rate</th>
<th>Protection %</th>
<th>Mean macroscopic lesion scoring in dead and sacrificed survivors (at 42 days of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Septic **</td>
</tr>
<tr>
<td>1</td>
<td>Treated/Non-vaccinated/Challenged</td>
<td>11</td>
<td>81.67</td>
<td>18.33</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Untreated/Vaccinated/Challenged</td>
<td>37</td>
<td>38.33</td>
<td>61.67</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Treated/Vaccinated/Challenged</td>
<td>48</td>
<td>20</td>
<td>80.00</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Untreated/Non-vaccinated/Challenged</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Treated/Vaccinated/Non-Challenged</td>
<td>60</td>
<td>0</td>
<td>100.00</td>
<td>0</td>
</tr>
</tbody>
</table>

*Total No. of birds/group = 60 ** Lesion score: (0) Absent, (1) Minimal, (2) Slight, (3) Moderate and (4) Severe lesions. *** Septic = Septiculosis.

### Table 2: Effect of peppermint and eucalyptus essential oils on body weight and FCR of different broiler chicken groups.

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Treatment</th>
<th>Experimental Days</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Treated/Non-vaccinated/Challenged</td>
<td>39.5±0.3</td>
<td>132.2±2.4</td>
<td>358.8±4.5</td>
<td>543.5±7.8</td>
<td>990.5±17.9</td>
<td>1212.1±13.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Untreated/Vaccinated/Challenged</td>
<td>39.7±0.3</td>
<td>127.5±2.6</td>
<td>350.5±5.8</td>
<td>580.9±11.3</td>
<td>1117.5±47.1</td>
<td>1282.1±23.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Treated/Vaccinated/Challenged</td>
<td>39.7±0.3</td>
<td>129.5±2.4</td>
<td>353.6±4.1</td>
<td>573.7±9.1</td>
<td>1123.0±41.9</td>
<td>1368.3±26.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Untreated/Non-vaccinated/Challenged</td>
<td>39.9±0.2</td>
<td>132.7±2.2</td>
<td>328.3±5.9</td>
<td>473.8±5.3</td>
<td>979.4±13.7</td>
<td>1077.5±18.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Treated/vaccinated/Non-Challenged</td>
<td>39.7±0.3</td>
<td>130.8±2.5</td>
<td>379.1±3.2</td>
<td>605.3±10.4</td>
<td>1095.5±15.9</td>
<td>1525.0±36.2</td>
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</tr>
</tbody>
</table>

Probability 0.7318 0.3256 0.0001 0.0001 0.0001 0.0001

### Feed Conversion Ratio (FCR)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experimental Days</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Treated/Non-vaccinated/Challenged</td>
<td>1.259±0.018</td>
<td>1.325±0.019</td>
<td>1.829±0.032</td>
<td>1.829±0.027</td>
<td>2.255±0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Untreated/Vaccinated/Challenged</td>
<td>1.311±0.029</td>
<td>1.338±0.019</td>
<td>1.807±0.029</td>
<td>1.772±0.024</td>
<td>2.382±0.027</td>
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<td></td>
</tr>
<tr>
<td>3 Treated/Vaccinated/Challenged</td>
<td>1.279±0.028</td>
<td>1.325±0.029</td>
<td>1.817±0.036</td>
<td>1.761±0.027</td>
<td>2.225±0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Untreated/Non-vaccinated/Challenged</td>
<td>1.232±0.028</td>
<td>1.346±0.031</td>
<td>1.932±0.043</td>
<td>1.768±0.031</td>
<td>2.460±0.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Treated/Non-vaccinated/Challenged</td>
<td>1.271±0.028</td>
<td>1.376±0.027</td>
<td>1.862±0.034</td>
<td>1.850±0.028</td>
<td>2.062±0.027</td>
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<td></td>
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</tbody>
</table>

Probability 0.0557 0.6176 0.1072 0.0732 0.0001

Mean±SD with different superscripts within column, within age, are significantly different (P≤0.05).
Table 3: Results of mean ELISA–conversion titers during first 4 weeks of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean ELISA-titers</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>7 days</td>
</tr>
<tr>
<td>1</td>
<td>Treated/Non-vaccinated/Challenged</td>
<td>15252±0.12</td>
</tr>
<tr>
<td></td>
<td>8371±0.234</td>
<td>3549±0.439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3737±0.409</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4590±1.06</td>
</tr>
<tr>
<td>2</td>
<td>Untreated/Vaccinated/Challenged</td>
<td>15252±0.23</td>
</tr>
<tr>
<td></td>
<td>8342±0.398</td>
<td>3586±0.577</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3494±1.14</td>
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<td></td>
<td></td>
<td>3950±0.11</td>
</tr>
<tr>
<td>3</td>
<td>Treated/Vaccinated/Challenged</td>
<td>15252±0.23</td>
</tr>
<tr>
<td></td>
<td>8451±0.312</td>
<td>3687±0.555</td>
</tr>
<tr>
<td></td>
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<td>3941±0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4879±1.01</td>
</tr>
<tr>
<td>4</td>
<td>Untreated/Non-vaccinated/Challenged</td>
<td>15252±0.23</td>
</tr>
<tr>
<td></td>
<td>7023±0.50</td>
<td>3367±0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1813±0.86</td>
</tr>
<tr>
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<td>1170±0.91</td>
</tr>
<tr>
<td>5</td>
<td>Treated/vaccinated/Non-Challenged</td>
<td>15252±0.23</td>
</tr>
<tr>
<td></td>
<td>8551±0.464</td>
<td>3693±0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3972±1.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4955±1.78</td>
</tr>
</tbody>
</table>

Mean±SD with different superscripts within column, within age, are significantly different (P≤0.05).

Table 4: Results of Haemagglutination-Inhibition (HI) seroconversion titers during first 4 weeks of age

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>HI-titers (Log_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>14 days</td>
</tr>
<tr>
<td>1</td>
<td>Treated/Non-vaccinated/Challenged</td>
<td>5.50±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.42±0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.83±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.92±0.11</td>
</tr>
<tr>
<td>2</td>
<td>Untreated/Vaccinated/Challenged</td>
<td>7.50±0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.75±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.00±0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.67±0.21</td>
</tr>
<tr>
<td>3</td>
<td>Treated/Vaccinated/Challenged</td>
<td>8.83±0.14</td>
</tr>
<tr>
<td></td>
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<td>8.00±0.14</td>
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<tr>
<td></td>
<td></td>
<td>8.08±0.19</td>
</tr>
<tr>
<td></td>
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<td>7.00±0.28</td>
</tr>
<tr>
<td>4</td>
<td>Untreated/Non-vaccinated/Challenged</td>
<td>5.42±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.00±0.23</td>
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<tr>
<td></td>
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<td>4.42±0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.50±0.11</td>
</tr>
<tr>
<td>5</td>
<td>Treated/vaccinated/Non-Challenged</td>
<td>8.33±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.25±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.08±0.28</td>
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<td>7.00±0.25</td>
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<td></td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Probability</td>
<td></td>
</tr>
</tbody>
</table>

Geometric mean maternal antibody titer at 1 day of age is 7.1. Mean±SD with different superscripts within column, within age, are significantly different (P≤0.05).

Fig. 1: PCR electrophoretic pattern of multiplied gene of fusion protein of NDV. Lane1: 100 base pairs molecular ladder. Lane2: Egg fluid sample showed +ve result at 200 bp. Lane3-5: Negative control sample for egg fluid sample.

Fig. 2: Amino acid sequence of the fusion protein of NDV isolate.

TGCAGCAATGCTCTCTTGACGGCAAGGAGGGCCTTTGCAAGGAAGGATTAACACGATCAAGGGGAGATACGTGAGAGAGAGATACGTAACACCTTTTTTTTTTTAAAACAGGGTCATCATAGTCATAAGTTGGCTTCTGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
DISCUSSION

The high survival rate (100%) in the negative control group (Treated/Vaccinated/Non-challenged) indicated the safety of the used ND vaccines and the administered essential oils blend. On the other hand; a 0% survival rate recorded in positive control group (Untreated/Non-vaccinated/Challenged) indicated the velogenicity of the used vVND virus. In challenged bird groups; the effect of volatile oils blend obtained in the present investigation might be due to their antiviral activity (Sökmen et al., 2004). This result confirms the findings of Awaad et al. (2002) on studying the effect of peppermint and eucalyptus essential oils on concomitant infection of "ORT" and "vVND" in broiler chickens. Our obtained findings are also in agreement with those reported by Barbour et al. (2013) who evaluated the impact of eucalyptus and peppermint essential oils on immune modulation and production of broiler chickens challenged with a molecularly characterized velogenic Newcastle disease virus. Findings of group 1 could be explained as this blend has a potent immunomodulatory effect that confirm findings of Awaad et al. (2009a) who stated that eucalyptus and peppermint essential oils blend implement both innate-cell mediated and humoral immune response. Similar findings has been reported by Barbour and Danker (2005) who mentioned that essential oils of eucalyptus and peppermint improved the homogeneity of immune responses and performance in Mycoplasma gallisepticum/H9N2 virus-infected broilers.

Essential oils supplementation significantly improved performance parameters (body weight and FCR) of the treated groups. These variables correlated positively with the survival percentage. The worst parameters has been recorded in positive control group 4 (Untreated/Non-vaccinated/Challenged). The improving in performance by essential oils supplementation has been described by other workers (Cross et al. 2007; Windisch et al., 2008; Barbur et al., 2013). The proposed mode of action of herbal products might be attributed to their antimicrobial properties (Kollanoor-Johny et al., 2012; Lee et al., 2013), oxidative-resistant activity (Dundar et al., 2008; Sarac et al., 2009) or anti-inflammatory effect (Lee et al., 2009; Yanhong, 2011). The significantly higher immune status in ND vaccinated/volatile oils treated groups 3 and 5 as well as non-vaccinated/volatile oils treated group 1 vs. positive control group 4 could be attributed to the fact that peppermint oil maintains the structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radical oxidants, thereby resulting in an improved immune response (Nickels, 1996). Awaad et al. (2009a) concluded that administration of eucalyptus and peppermint oils blend evoked the immune response in chickens. Enhancement of the immune system by herbal products and oils has been reported by many investigators (Lee et al., 2010; Lillehoj et al., 2010).

The improvement in vVND pathological picture (macroscopic and microscopic lesion scores) recorded in volatile oils supplemented groups (with or without vaccination) is supposed to their possible antiviral (virusidal) action that might influenced the virulence of the challenging agent (Mekay and Blumberg, 2006; Ocak et al., 2008; Barbour et al., 2010). Moreover; this perhaps attributed also to their effect on cell mediated and local immunity (Cserep, 2008) which has been shown to play a part in clearing virus from the tissues (Awaad et al., 2009a). The recorded increase in the follicular size comprising the cecal tonsils with lymphohlastic activation of follicular elements in groups 1, 3 and 5 completely accords with those reported by Awaad et al. (2009a) who mentioned that treated birds with peppermint and eucalyptus essential oils resulted in lymphocytic hyperplasia and activation in Bursa of Fabricious, thymus, spleen and caecal tonsils.

Conclusions: The used peppermint and eucalyptus essential oils blend proved its immunomodulatory effects and positively evoked the immune response against vVND virus that have a great devastating economic impact on poultry industry. It also improved clinical, pathological and performance parameters.

### Table 5: Statistical analysis of histopathological lesion scoring of vVND challenged broiler chickens

<table>
<thead>
<tr>
<th>Histopathological lesions</th>
<th>vVND challenged groups</th>
<th>Trachea</th>
<th>Lungs</th>
<th>Cecal tonsils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1*</td>
<td>G2</td>
<td>G3</td>
<td>G4</td>
</tr>
<tr>
<td>Deciliation &amp; Desquamation &amp; necrosis</td>
<td>2.00±0.31**</td>
<td>1.75±0.47**</td>
<td>1.50±0.28**</td>
<td>3.00±0.57**</td>
</tr>
<tr>
<td>Inflammatory reaction</td>
<td>1.40±0.40**</td>
<td>1.80±0.37**</td>
<td>1.25±0.25**</td>
<td>2.75±0.74**</td>
</tr>
<tr>
<td>Congestion</td>
<td>1.66±0.66**</td>
<td>1.75±0.25**</td>
<td>1.40±0.24**</td>
<td>2.66±0.66**</td>
</tr>
<tr>
<td>Edema</td>
<td>1.66±0.66**</td>
<td>1.50±0.28**</td>
<td>1.40±0.24**</td>
<td>2.50±0.50**</td>
</tr>
<tr>
<td>Lungs</td>
<td>a-Secretory bronchi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desquamation &amp; necrosis</td>
<td>1.00±0.40**</td>
<td>2.70±0.80**</td>
<td>0.80±0.20**</td>
<td>3.00±0.31**</td>
</tr>
<tr>
<td>Inflammatory reaction</td>
<td>2.00±0.40**</td>
<td>2.00±0.83**</td>
<td>1.25±0.25**</td>
<td>2.80±0.37**</td>
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<tr>
<td>Congestion</td>
<td>1.40±0.54**</td>
<td>1.80±0.64**</td>
<td>1.16±0.40**</td>
<td>2.60±1.14**</td>
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<tr>
<td>Edema</td>
<td>1.40±0.24**</td>
<td>1.80±0.73**</td>
<td>1.25±0.25**</td>
<td>2.40±0.50**</td>
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<tr>
<td>Lungs</td>
<td>b-Parabronchi &amp; air capillaries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desquamation &amp; necrosis</td>
<td>1.10±0.23**</td>
<td>1.64±0.25**</td>
<td>0.72±0.19**</td>
<td>2.33±0.25**</td>
</tr>
<tr>
<td>Inflammatory reaction</td>
<td>1.00±0.25**</td>
<td>1.23±0.35**</td>
<td>0.86±0.16**</td>
<td>2.86±0.29**</td>
</tr>
<tr>
<td>Congestion</td>
<td>1.75±0.16**</td>
<td>1.66±0.31**</td>
<td>1.13±0.16**</td>
<td>2.82±0.26**</td>
</tr>
<tr>
<td>Intestinal and perivascular edema</td>
<td>1.60±0.26**</td>
<td>1.47±0.35**</td>
<td>1.14±0.14**</td>
<td>2.29±0.31**</td>
</tr>
<tr>
<td>Cecal tonsils</td>
<td>1.25±0.25**</td>
<td>1.00±0.00 b</td>
<td>1.00±0.00 b</td>
<td>1.83±0.30**</td>
</tr>
</tbody>
</table>

*G1 = Treated Non-vaccinated, G2 = Untreated Vaccinated, G3 = Treated Vaccinated, G4 = Untreated Non-vaccinated (Positive control), Mean±SD with different superscripts within row, within age, are significantly different (P≤0.05). No lesions could be detected in group 5.
Fig. 3: Microscopic lesions in trachea. H & E X200. a) Mentofin treated group (group 1) showing deciliation and hyperplasia of tracheal mucosal epithelium associated with lymphocytic infiltration and congestion with mild edema of lamina propria with few RBCs exudation (score 1). b) Vaccinated group (group 2) showing hypertrophy and hyperplasia of tracheal mucosa with extensive deciliation and lymphocytic infiltration with moderate congestion and edema. c) Mentofin treated vaccinated group (group 3) showing focal mild deciliation of tracheal mucosa with goblet cell hyperplasia and mild inflammatory reaction involving the mucosa. d) Untreated non-vaccinated (positive control) group (group 4) showing extensive necrosis with hyperplasia of tracheal mucosa with severe edema, congestion and hemorrhages in lamina propria.

Fig. 5: Microscopic lesions of caecal tonsils. H & E. a) Vaccinated group (group 2) showing extensive hyperplasia of the lymphoid follicles (X100). b) Mentofin treated group (group 1) showing mild depletion of lymphoid elements (X400). c) Mentofin treated vaccinated group (group 3) showing mild lymphocytic depletion of lymphoid follicle with lymphoblastic activation (arrow) (X400). d) Untreated non-vaccinated (positive control) group (group 4) showing severe lymphocytolysis and necrosis of majority of lymphoid elements comprising the follicle (X400).

REFERENCES


